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The regenerative potential of mammary gland stem cells may be exploited to regulate milk production in dairy cattle. Our recent works demonstrated the intrinsic regenerative potential found in bovine mammary tissue, but a greater challenge is to purify and enrich stem cells that reside in the mammary gland. We describe here the phenotype of mammary epithelial cells emerging in two defined culture conditions and the assessment of their in vivo regenerative potential after long-term culture. We used Epicult B and SF7 media (both are used to culture mouse and human mammary epithelial cells) to expand bovine cells. We analyzed cell phenotype at different passages (P0, 1, 3 and 5; cells were passaged every 5 days). We also tested the regenerative potential of cells cultured in different media through Colony Forming Cell assay (CFC) and through xenotrasplantation in NOD/SCID mice. Cytokeratin 18 (CK18) and cytokeratin 14 (K14) were observed in both media until P5. Significant differences in marker expression were found that were associated with the medium used. Vimentin (a stromal marker) was differently expressed in EpiB and SF7 at P5 (0% for EpiB and 2% for SF7, $p < 0.05$). K14 and K18, P63 and EpCam expression was also evaluated in regenerated polarized structures found in NOD/SCID mice. These results demonstrate that a long-term culture is influenced by the medium used but maintains a multi-potent cell subpopulation with intrinsic regenerative potential.
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